Title: Brain choline concentration: early quantitative marker of ischemia and infarct expansion?

B Karaszewski[1,2,3], MD (e-mail: bartosz@karaszewski.org)

RGR Thomas[1,2], MRCP (e-mail: ralph.thomas@nhs.net)

FM Chappell[1], PhD (e-mail: francesca.chappell@ed.ac.uk)

PA Armitage[1,2], PhD (e-mail: paul.armitage@ed.ac.uk)

TK Carpenter[1,2], PhD (e-mal: trevor.carpenter@ed.ac.uk)

GKS Lymer[1,2], PhD (e-mail: katherine.lymer@ed.ac.uk)

MS Dennis[2], MD (e-mail: martin.dennis@ed.ac.uk)

I Marshall[1,4], PhD (e-mail: ian.marshall@ed.ac.uk)

JM Wardlaw[1,2], FRCR (e-mail: Joanna.Wardlaw@ed.ac.uk)

[1]SFC Brain Imaging Research Centre, SINAPSE Collaboration,

[2]Division of Clinical Neurosciences, University of Edinburgh, UK

[3]Department of Neurology of Adults, Medical University of Gdansk, Poland

[4]Medical Physics & Medical Engineering, Medical & Radiological Sciences, University of Edinburgh, UK

Correspondence: Bartosz Karaszewski, MD, PhD

SFC Brain Imaging Research Centre, SINAPSE Collaboration, Division of Clinical Neurosciences, The University of Edinburgh, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK

Tel.: +44 – 1315372943, Fax: +44 - 1313325150

E-mail: bartosz@karaszewski.org

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Abstract

Objective: Better prediction of tissue prognosis in acute stroke might improve treatment decisions. We hypothesized that there are metabolic ischemic disturbances measurable non-invasively by proton MR spectroscopy (^{1}HMRS) that occur earlier than any structural changes visible on diffusion tensor imaging (DTI), which may therefore serve for territorial identification of "tissue at risk". **Methods:** We performed multi-voxel ^{1}HMRS plus DTI within a maximum of 26 hours, and DTI at three-seven days, after ischemic stroke. We compared choline, lactate, NAA, creatine concentrations in normal-appearing voxels that became infarcted("infarct expansion"), with normal-appearing voxels around the infarct that remained "healthy"("non-expansion") on follow-up DTI. Each "infarct expansion" voxel was additionally classified as either "complete infarct expansion"(infarcted tissue on follow-up DTI covered ≥50% of the voxel) or "partial infarct expansion"(<50% of voxel). **Results:** In 31 patients (NIHSS:0–28) there were 108 infarct "non-expansion" voxels and 113 infarct "expansion" voxels (of which 80 were "complete expansion" and 33 "partial expansion" voxels). Brain choline concentration increased for each change in expansion category from "non-expansion", via "partial expansion" to "complete expansion" (2423, 3843, 4158i.u.; p<0.05). Changes in lactate, NAA and creatine concentrations in expansion category were insignificant although for lactate there was a tendency to such association. **Conclusions:** Choline concentration measurable with ^{1}HMRS was elevated in peri-ischemic normal-appearing brain that became infarcted by three-seven days. The degree of elevation was associated with the amount of infarct expansion. ^{1}HMRS might identify DTI-normal appearing tissue at risk of conversion to infarction in early stroke.

Introduction

In clinical stroke practice, management decisions, including use of thrombolytic treatment, are based on physical examination, neuroimaging to exclude hemorrhage, and time from stroke onset to treatment, assuming that there is "tissue at risk" of infarction that could be salvaged. However, for some patients, very early time windows are already too late, whilst others may have salvageable tissue for many hours after stroke, making the time window alone too non-specific[1,2].

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Currently there is no reliable, sensitive and specific method for early non-invasive determination of tissue at risk: the mismatch between Diffusion- and Perfusion-Weighted Imaging is still being evaluated[1-4], and there are no CSF or blood markers that diagnose stroke or predict prognosis reliably[5,6].

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We hypothesized that in acute ischemic stroke, metabolic disturbances could be measurable in ischemic tissue much earlier than any structural changes. If true, changes in brain metabolite concentrations in normal appearing tissue on diffusion imaging outside the lesion soon after ischemic stroke measured with Magnetic Resonance Spectroscopy (MRS)[7,8] might predict the likelihood and direction of further infarct expansion and hence prognosis. To test this hypothesis, we compared concentrations of selected brain metabolites measured with MRS in the normal-appearing tissue around the acute infarct as seen on Diffusion Tensor Imaging (DTI) early after stroke that converted to infarction by three to seven days, with that which remained normal on DTI. The selection of metabolites for this study was based on the assumptions that they should represent important elements in pathophysiological pathways following acute stroke and that their concentrations are readily observable in a short scanning time.

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Methods

Patient Recruitment

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We prospectively recruited patients with acute ischemic stroke and without contraindications to Magnetic Resonance Imaging (MRI) admitted to our hospital acute stroke service. Each patient was carefully examined by a stroke physician who measured stroke severity using National Institutes of Health Stroke Scale (NIHSS) and determined stroke sub-type by the Oxfordshire Community Stroke Project (OCSP) classification[9]. Patients underwent MRI as soon as possible after stroke but within a maximum of 24 hours from onset. Follow-up MRI was performed at three to seven days after stroke. Onset was defined as the time when signs were first noticed by a patient, or symptoms first observed, or, if a patient awoke already having stroke symptoms, the time last known to be well.

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Standard Protocol Approvals, Registrations, and Patient Consents

The study was approved by the Lothian Research Ethics Committee on human experimentation and written informed consent was obtained from the patients or assent from their relatives.

Diffusion and Spectroscopy Techniques

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All MR data were obtained on a GE Signa HDX 1.5T (General Electric, Milwaukee, WI, USA) scanner with self-shielding gradients (33 mT/m maximum) and a ‘birdcage’ quadrature head coil. In each patient we performed axial T_2-weighted fast spin-echo and FLuid Attenuated Inversion Recovery (FLAIR) imaging, axial DWI and/or DTI with field-of-view (FOV) 240x240 mm, 15 axial slices of thickness 5 mm, slice gap 1 mm, acquisition matrix 128x128, echo time 97.4 ms, repetition time 10 s and diffusion

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sensitizing gradients with scalar b-values of 1000 s/mm^2 applied in six non-collinear directions, and Multi-voxel Point Resolved Spectroscopy (PRESS)-localized proton MRS (^{1}HMRS, FOV 320x320 mm, slice thickness 10 mm, acquisition matrix 24x24, echo time 145 ms and repetition time 1000 ms). DTI and ^{1}HMRS with FLAIR and T^{2*} imaging were performed on admission, and DTI, FLAIR, and T^{2*} at three-seven days after stroke. The ^{1}HMRS voxel grid was carefully centered on the slice showing the maximum ischemic lesion extent on DTI (Figures 1 and 2) and placed within brain to avoid contamination of the spectra by lipid signal from bone marrow or subcutaneous tissue, but to include as much as possible of the brain as possible. We used the scanner's standard three-pulse CHEmical Shift Selective (CHESS) water suppression and shimming, optimized on the slice of interest. Additional saturation bands were placed around the PRESS box to minimize lipid contamination. Each ^{1}HMRS data set took approximately nine minutes to acquire, and the data were effectively 'averaged' over this period. Bulk patient motion and eddy current-induced artifacts were removed from the DTI data using a three dimensional (3D) computational image alignment program to register the component echo-planar imaging volumes to the T_2-weighted volumes acquired with the DTI protocol. Maps of the average DTI signal were obtained from the six DTI images acquired for each slice.

Spectroscopic images were interpolated to a 32x32 matrix yielding 1000 mm^3 voxels and all processing was carried out on a voxel-by-voxel basis after setting the residual water signal in each voxel to a standard chemical shift of 4.70 ppm. All spectroscopic data were modelled in the time domain by five Gaussian components (corresponding to choline, creatine, N-acetyl aspartate (NAA) and the lactate doublet) using the Advanced Method for Accurate Robust and Efficient Spectral Fitting (AMARES)

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algorithm within the Magnetic Resonance User Interface (MRUI) package (http://www.mrui.uab.es/mrui). The data were transformed to spectra for display and visual quality control purposes.

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Metabolite Concentrations

Choline, creatine, NAA and lactate were identified by their characteristic appearances at echo time 145 ms (Figure 1). Metabolite quantification took into account coil loading (using the scanner's radiofrequency transmitter gain) and receiver gain thus enabling inter-subject (and obviously intra-subject) comparison of individual metabolite concentrations. Careful patient set-up ensured between subject set-up reproducibility and good coil uniformity: we previously found that coil uniformity is very good across an axial slice near the centre of the coil (data not published). Our metabolite concentration unit was an 'institutional unit' (i.u.).

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Tissue Classification and Estimation of Tissues Metabolites' Concentrations

^{1}HMRS and DTI data were co-registered using an affine transformation. The multi-voxel MRS grid was superimposed onto the admission DTI using software designed in-house. The grid voxels on admission DTI were classified as falling on or outside the acute DTI hyperintense ischemic lesion blind to all other information; then the voxel grid was compared with the DTI lesion appearance at three-seven days to identify voxels superimposed on tissue located outside the lesion that were normal on admission and remained normal on follow-up, or voxels superimposed on tissue located outside the lesion that were normal on admission but became hyperintense on DTI at three-seven days, also blind to all other data. We used the diffusion image at three-seven days as an indication of infarct growth because at three-seven days

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diffusion imaging usually mirrors the three-seven day T2 or FLAIR appearance[10], and is easier to assess visually than the B0-T2 image. Although the three-seven day infarct extent is not the final infarct (because infarct evolution is generally considered to be complete by three months), it is a useful indicator of early lesion growth due to recruitment of early penumbral tissue into the infarct before other secondary events can influence infarct extent (e.g. recurrent stroke, hypotension).

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The concentrations of choline, creatine, NAA and lactate from each voxel in the spectroscopy grid were extracted and compared between: 1) voxels on the admission DTI that appeared normal around the ischemic lesion, but that converted to infarction on the follow-up DTI ("infarct expansion" voxels) and 2) voxels on the admission DTI that appeared normal around the ischemic lesion, which remained "healthy" on the follow-up DTI ("non-expansion" voxels).

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Additionally, each "infarct expansion" voxel was classified as either "complete infarct expansion" voxel (infarcted tissue on the follow-up DTI covered over 50% of each individual voxel) or "partial infarct expansion" voxel (infarcted tissue on the follow-up DTI covered less than 50% of each individual voxel); Figure 2. The subdivision was introduced to investigate whether the metabolite concentration measured early after stroke in peri-infarcted tissue can be used for estimating the magnitude of potential infarct expansion (quantitative analysis) in addition to making prognosis on infarct expansion on a "yes" or "no" basis.

Statistical Analysis

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We compared choline, creatine, NAA and lactate concentrations in "non-expansion" and "expansion" voxels using a linear mixed model. We tested the change in metabolite concentrations per change in expansion category: from "non-expansion", via "partial expansion” (below 50% of each voxel), to “complete expansion” (over 50% of each voxel). We used the logarithmic transformations (log) of the metabolite concentrations rather than their raw values because the model fitted better when metabolite concentrations were linear.

Results

We recruited 31 patients (mean age: 74 years; range: 45-88) with acute ischemic stroke between December 2007 and March 2009 with admission and follow-up imaging. According to OCSP classification[9] there were 11 TACS, 12 PACS, 5 LACS, 2 POCS, and 1 of undetermined subtype. The mean NIHSS was 10, median 7, range: 0-28. The mean time from stroke onset to admission MRI was 16.3 hours, median 17 hours, range four to 26 hours. Four patients underwent initial MRI by two hours beyond the designed 24 hours but were included in the analysis (decision taken blind to any information on the results) as the benefits (more reliable statistics in a larger cohort) outweighed the drawbacks. We identified 108 "non-expansion” voxels and 113 "expansion” voxels amongst which there were 80 “complete expansion” voxels and 33 “partial expansion” voxels. Amid 31 patients there were 27 with at least one “non-expansion” voxel and 20 with one or more “expansion” voxels. Among five patients classified as LACS on admission, definite lesion growth was observed in three using our voxel classification.

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Choline

Mean brain choline concentration in "healthy-looking" voxels on initial DTI that converted to infarction on the follow-up DTI (“expansion” voxels) was higher than in DTI tissues immediately outside the lesion which remained “healthy-looking” on the follow-up DTI ("non-expansion" voxels; Figure 3); the log of the choline concentration increased by 0.48 units (95% CI 0.0069, 0.95) for each change in expansion category from the baseline of "non-expansion", through "partial" to "complete" expansion, (p=0.047; Figure 3).

Lactate

Although lactate concentration was highest in "complete expansion" voxels and lowest in "non-expansion" voxels ("complete expansion": 1078; "partial expansion": 962; "non-expansion": 654 i.u.), the change in tissue lactate concentration with "expansion" category was not significant (Figure 4).

N-acetyl Aspartate

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There was no difference in NAA concentration in "expansion" versus "non-expansion" voxels (p=0.60; Figure 4).

Creatine

There was no difference in creatine concentration in "expansion" versus "non-expansion" voxels (p=0.13; Figure 4).

Discussion

We found that choline concentration measured by ^{1}HMRS in DTI-normal appearing tissue located around the ischemic lesion within the first 26 hours from stroke onset was elevated in those voxels that became infarcted on DTI within the next few days. Moreover, the degree of ischemic expansion was associated with the degree of elevation of choline concentration. Further studies are required to determine whether choline concentration is a reliable, sensitive or specific measure for predicting infarct growth, including identification of the potential threshold values, and therefore could be used to support treatment decisions in routine practice.

We speculated that metabolite concentrations might have prognostic value because some metabolic products are pathophysiologically likely to mirror cellular sufficiency/insufficiency at the time of imaging. This is in contrast to other approaches to detect “tissue at risk”, for example perfusion imaging (including DWI/PWI mismatch), which gives an indirect estimate of tissue state by extrapolating from blood flow levels[11,12]

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Hypothetically, in ischemic stroke patients, thrombolytic treatment might be considered over six hours from onset if there was still a high probability of further lesion expansion and if this could be assessed reliably and non-invasively on admission. However, as this is only speculative at this stage, further studies are required involving prospective ^{1}HMRS metabolite measurement in acute ischemic stroke with clinical characteristics, ideally in patients treated with tissue plasminogen activator (tPA). Importantly, the time needed to obtain ^{1}HMRS data seems acceptable in clinical setting as it takes not longer than ten minutes (much less in some circumstances). Although currently our approach demands specialist off line image processing, all MR manufacturers now provide MRS processing software on console, which could be used in routine practice enabling results to be obtained within reasonable time after MRI.

Several previous studies assessed metabolite concentrations measured by MRS to predict final infarct size, clinical deficit or functional outcome[11-16]. However, with few exceptions[11,12,16], they mainly focused on metabolites measured within the lesion core. In our study we prospectively investigated associations between metabolic

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We selected lactate, NAA, choline and creatine because their concentrations are readily observable in a relatively short scanning time using a widely available MRS technique. The short scanning time is important in managing severely ill stroke patients as it does not significantly delay interventions in clinical practice[7,8]. Concentrations of other metabolites measurable with longer scanning sessions (such as glutamate) might be of support in estimating "penumbra" but are currently much more difficult to measure reliably in clinical practice, although their utility for this approach should be also investigated in the future.

Previous studies on choline pathophysiology in the brain showed that mild hypoxia significantly increased cerebral choline levels[17] and its concentration decreased within days of ischemia-induced membrane rupture[18]. The effect of mild hypoxia would be consistent with choline concentration being highest in the tissue which became infarcted, as in our results, although it is unlikely that membrane rupture would fit with our results as this is usually a late occurrence in established infarction when the lesion would be clearly visible on DTI, not in normal-appearing tissue. The hypotheses that might explain our finding of elevated choline and progression of penumbral tissue to infarction include upregulation of genes for enzymes responsible for metabolism of free choline, phosphatidylcholine (PC; a compound containing 95% of the total pool of body choline), phosphocholine or CDP-choline (intermediates towards PC synthesis), such as CTP:phosphocholine cytidylyltransferase, or body

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choline redistribution for the benefit of the ischemic brain[19]. These mechanisms might stimulate and enable neurite branching or stabilization of neurolemma, and thus promote tissue salvage[19]. Alternatively, patients whose ischemic lesion grew might have had lower brain choline concentrations before stroke, and therefore their brains' cells were prone to quicker membrane destabilization and thus to infarction.

We recently showed that choline is not a "stable" metabolite in acute stroke lesions, but changes with time after stroke[20]. This questioned the commonly regarded potential of choline to serve as a denominator for other metabolite concentration measurements, which may perhaps be the main reason for missing it as a marker of tissue at risk of infarction in its own right. Future studies are needed to explain the pathophysiological background of elevated choline concentration early after acute ischemia prior to any structural changes in the brain on DTI.

The increase in lactate concentration is the net effect of its overproduction, impaired utilization and decreased washout in ischemic brain and might correlate with oxygen deprivation[8,12,21-24]. In stroke patients, lactate concentration is the highest in the lesion core, and then in the penumbral tissue as defined by DWI/PWI mismatch[11,12] or in a rim of normal tissue on DTI immediately outside the lesion[8]. In previous studies[11,12] lactate correlated with the apparent diffusion co-efficient (ADC) and perfusion values but not NAA, thus confirming that all of these measures are markers of the presence of ischemia though not necessarily of tissue fate. In our cohort, brain lactate concentration increased from "non-expansion" through "partial expansion", to "complete expansion" tissue but the differences were not significant. Therefore a larger study is needed to properly assess the role of lactate in prediction of future infarction.

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NAA, a compound nearly exclusively localised in adult neurons decreases in ischemic stroke, the level of reduction being related to the severity of ischemia[12,14,25-30]. It is considered as a marker of neuronal death but also of tissue dysfunction[26,27]. However, in agreement with previous studies[12], we found that NAA concentration did not identify “active penumbra” in acute stroke. This is explainable physiologically because our metabolite measurements were performed when all analysed tissues were radiologically normal on DTI, prior to neuronal death. These results are also consistent with previous findings showing that although NAA was decreased in the lesion core, its concentration remained normal outside the visible infarct in penumbral tissue[11,12,16,25,28-30].

We had hypothesized that decreased brain concentrations of creatine might be associated with conversion to infarction because creatine inhibits caspase-mediated neuronal death[31,32]. However, we did not find that ^{1}HMRS-measured creatine concentrations identified “tissue at risk”, which might also question its putative neuroprotective role[31,32].

This study has several limitations. We were only able to recruit and repeat imaging on 31 subjects, a relatively small patient cohort. We have not compared metabolite concentrations with cerebral perfusion data which might be helpful to explain the pathophysiology background for some of our results. For example, elevated lactate in normal appearing voxels immediately outside the infarct would be consistent with a perfusion deficit (i.e. DWI/PWI mismatch) as in previous studies[12,14].

We compared the images from different time points voxel-by-voxel manually, a time-consuming and demanding procedure, and in some patients we did not manage to obtain identical admission and follow-up brain sections. Future developments might automate this procedure to better compensate for differences in head placement and

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image location between visits. We did not divide patients according to the time from stroke onset to imaging, and this may have influenced the results as tissue biochemical processes are much more dynamic than changes in structure. However, any subdivision of a 31-subject cohort would not produce reliable statistics. Additionally, the method for determining "tissue at risk" should be usable at any time from stroke onset. On the other hand, there might be different ranges of brain metabolite concentrations indicating the likelihood of conversion to infarction at different time points from stroke. Finally, the *in vivo* [1]HMRS has several physical limitations, as listed and discussed in detail previously[8].

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Figure legends:

Figure 1. Examples of ^{1}HMRS spectra from different brain regions defined on DTI of an acute ischemic stroke patient: lesion core (0400), infarct expansion area (0398), contralateral normal tissue (0561 and 0622).

Figure 2. Example of spectroscopy voxel categories according to the appearance of the brain on DTI and the changes of its appearance on the follow-up DTI. The voxel grid numbering on the follow-up is not consistent with the one on the admission image.

Figure 3. Gradual increase in brain choline concentration (mean of patients' means shown) with DTI infarct "expansion" characteristics ($p<0.05$).

Figure 4. Brain concentration of metabolites (mean of patients' means shown) in infarct "non-expansion" versus infarct "expansion" voxels (differences were not significant for lactate, NAA and creatine: $p>0.05$).

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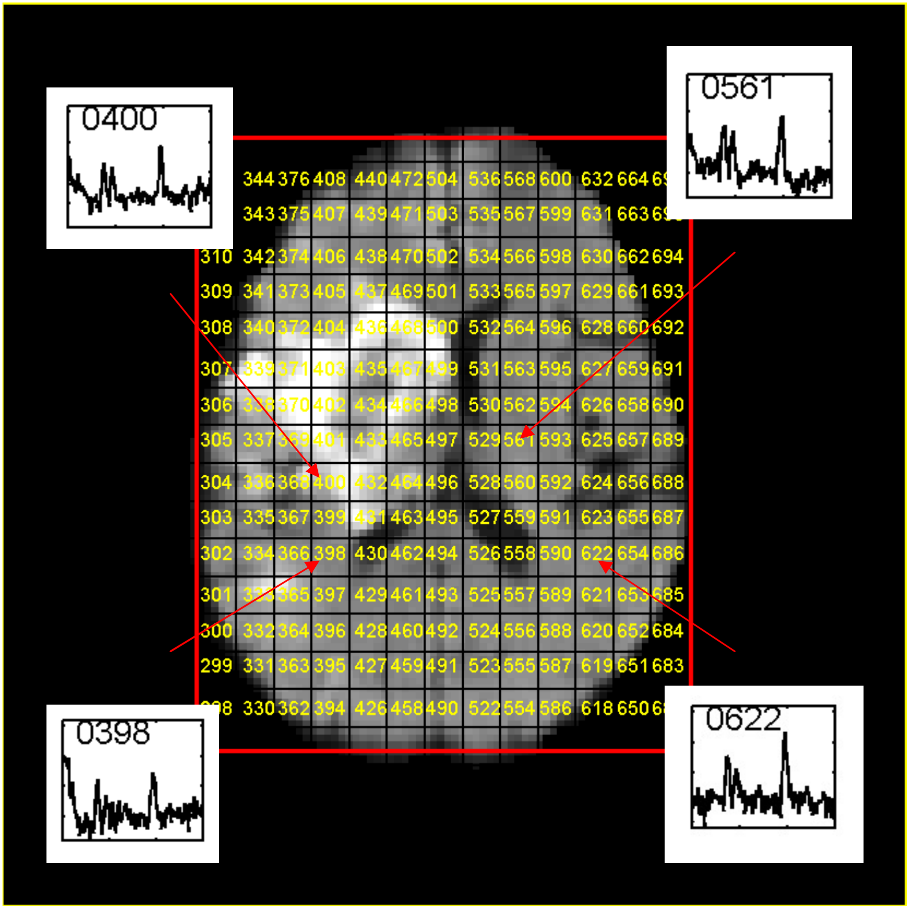


Figure 2.

ADMISSION **FOLLOW-UP**

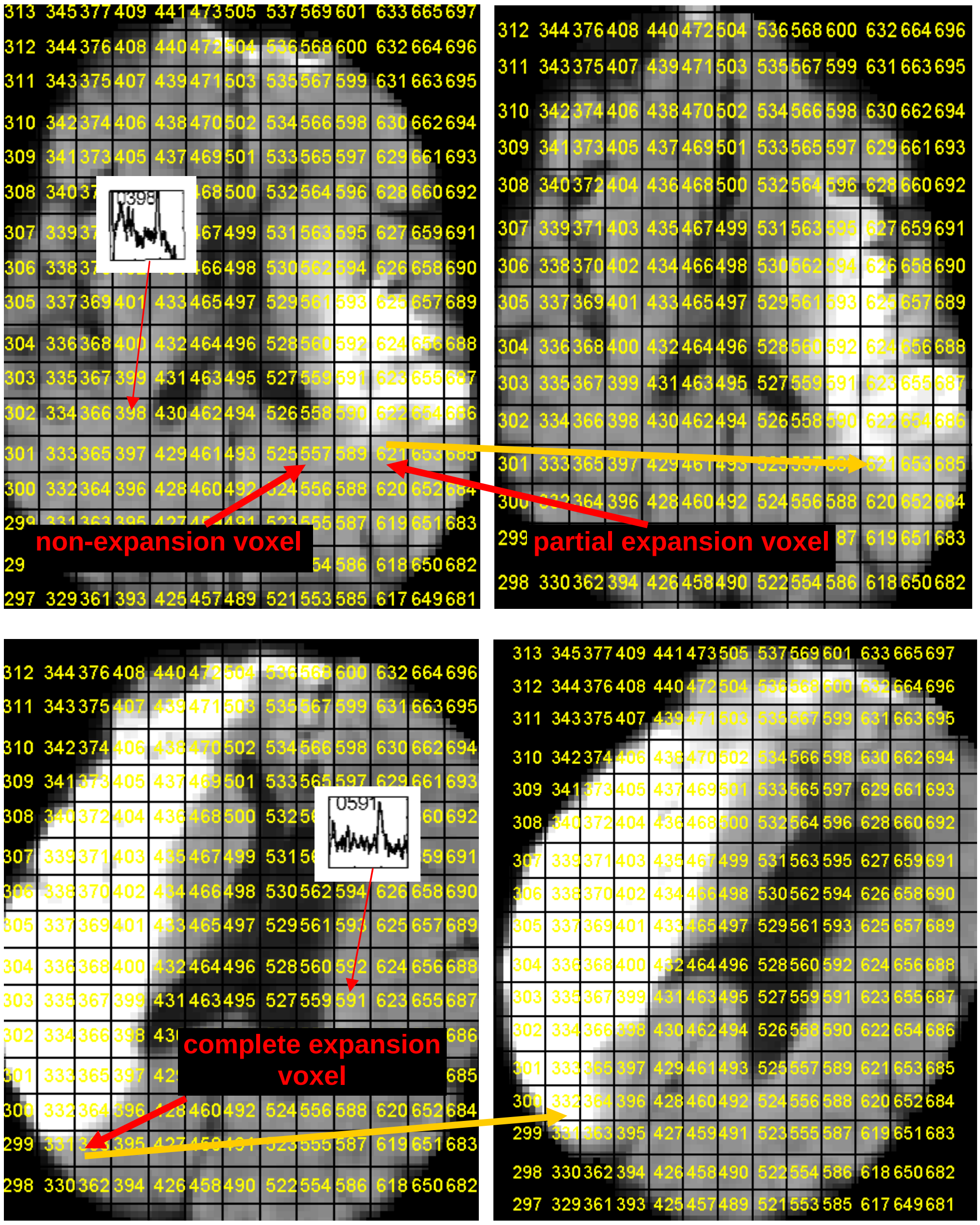


Figure 3. Gradual increase in brain choline concentration (mean of patients' means shown) with DTI infarct "expansion" characteristics ($p<0.05$).

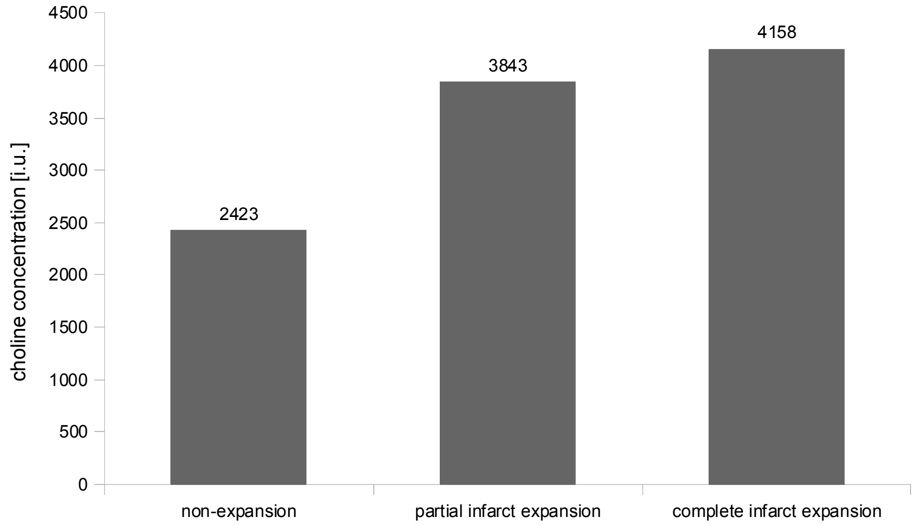
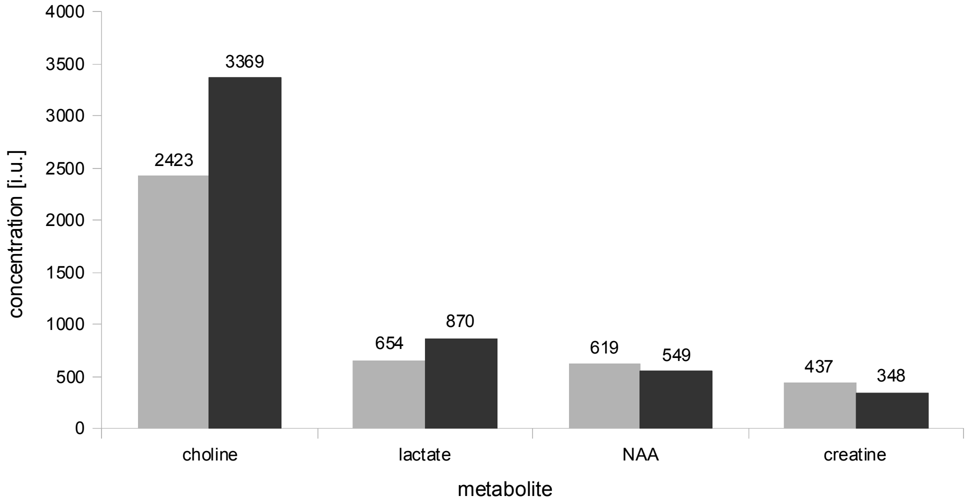


Figure 4. Brain concentration of metabolites (mean of patients' means shown) in infarct "non-expansion" (light columns) versus infarct "expansion" (dark columns) voxels (differences were not significant for lactate, NAA and creatine).



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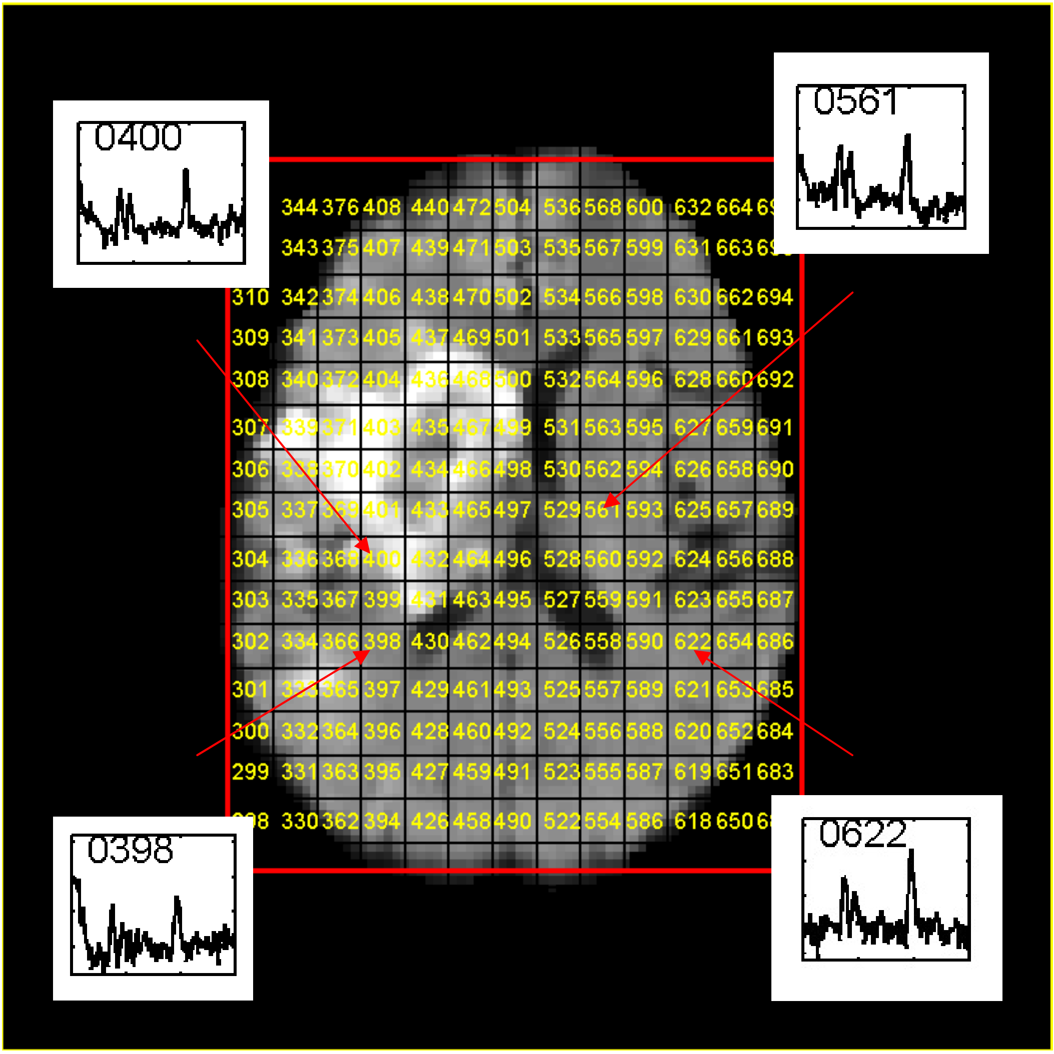


Figure 2.

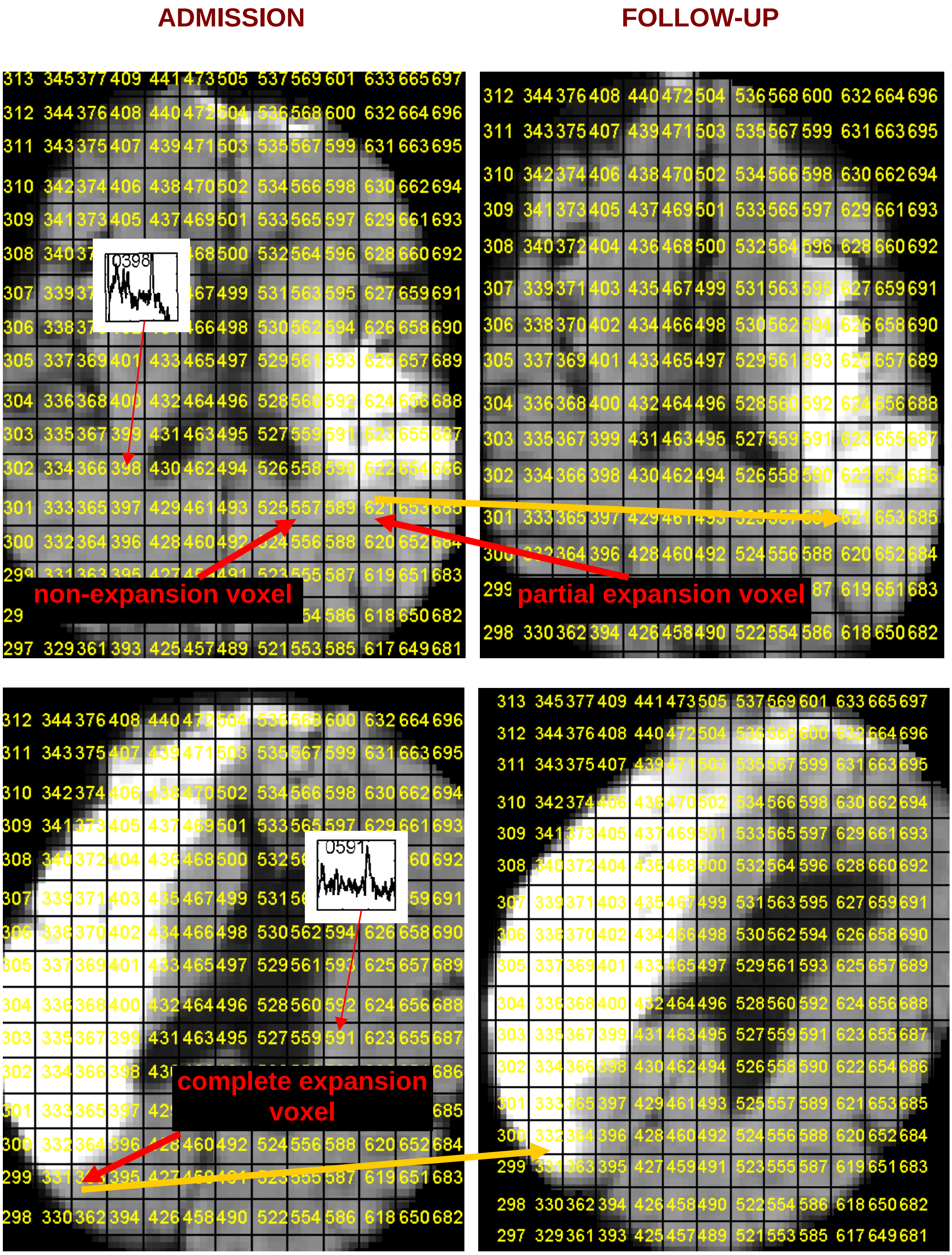
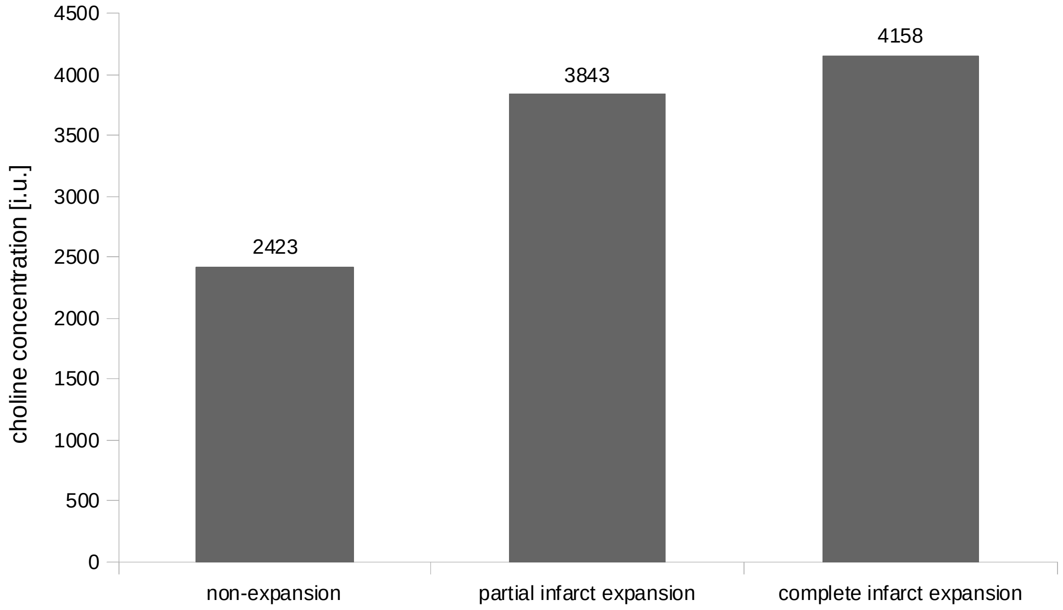

ADMISSION
FOLLOW-UP
0398
non-expansion voxel
partial expansion voxel
0591
complete expansion voxel

Figure 3. Gradual increase in brain choline concentration (mean of patients' means shown) with DTI infarct "expansion" characteristics ($p<0.05$).



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